EVALUATION OF THE ANTI-INFLAMMATORY ACTIVITY OF COMBINED SAMPA-SAMPALUKAN (*Phyllanthus niruri*) AND MAKAHIYA (*Mimosa pudica*) ETHANOLIC STEM EXTRACTS

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ABSTRACT

Inflammation is a localized reaction that manifests redness, swelling, warmth and pain after an injury or infection. This research aimed to find out if the combination of Sampa-Sampalukan (*Phyllanthus niruri*) and Makahiya (*Mimosa pudica*) ethanolic stem extracts effectively possess anti- inflammatory activity using carrageenan induced paw edema in male wistar rats (*Rattus novegicus*). Before one hour of injecting 0.1mL of 1% carrageenan, the rats were administered with different treatments: Group 1 received distilled water as the negative control, Group 2 was treated with Mefenamic acid Suspension as the positive control, Group 3 was treated with Sampa- Sampalukan (*P. niruri*), Group 4 was treated with Makahiya (*M. pudica*) and Group 5 was treated with the combination of Sampa-Sampalukan (*P. niruri*) and Makahiya (*M. pudica*) ethanolic stem extracts. The statistical significance of differences of the different groups was analyzed using paired t-test. The study demonstrated that combination of Sampa-Sampalukan (*P. niruri*) and Makahiya (*M. pudica*) ethanolic stem extracts in pre-treated rats has significantly decreased in paw size. Also, Sampa-Sampalukan (*P. niruri*), Makahiya (*M. pudica*) and the Combination of the stems showed the same effectiveness as with the Positive Control Mefenamic Acid Suspension. The findings from the above study prove that the combination of Sampa-Sampalukan (*P. niruri*) and Makahiya (*M. pudica*) ethanolic stem extracts has anti-inflammatory effect and can be an alternative for management of inflammation.

Key words: *Phyllanthus niruri*, *Mimosa pudica*, *anti-inflammatory, paw edema*

INTRODUCTION

Inflammation is part of the body's immune response. It is the body's ability to heal itself after an injury and protect itself against foreign invaders, such as viruses and bacteria; repair damaged tissue. The wounds will become infected and form pus, without inflammation it could be deadly. Inflammation can be problematic, though, it has a function in some chronic diseases (Szalay, 2015). If the inflammation becomes severe, it may result to general reactions in the body. General symptoms are exhaustion and fever. These are signs that the immune defense is very active and needs a lot of energy, which may be lacking for other activities.

Arthritis is one of the main leading causes of death in the U.S. (CDC, 2016, in excess of 50 million adults in the United States have been diagnosed to have some kind of Arthritis. Among adults with Arthritis, the predominance of serious joint pain will be 27 percent, with the most elevated commonness among people 45 to 64 years of age (31 percent) (Barbour, 2016). Arthritis is also common among adults who are obese than among those who are normal weight or underweight (Barbour, 2013). The annual mortality rate per 100,000 people from Rheumatoid Arthritis in the Philippines has increased by 17.6% since 1990, an average of 0.8% a year. For men, mortality from Rheumatoid Arthritis in the Philippines is at about age 70 to 74. It kills men at lowest rate at age 30 to 34. Women are killed at the highest rate from Rheumatoid Arthritis in the Philippines at age 80 plus. It will be least deadly to women at age 35 to 39. At 16.7 deaths per 100,000 women in 2013, the peak mortality rate for women will be higher than that of men, which will be 4.5 per 100,000 men (Siegel, Miller, & Jemal, 2017).

Non-steroidal Anti-inflammatory Drugs (NSAIDs) are regularly utilized pharmaceuticals to alleviate the inflammation. NSAIDs are cheap and regularly recommended for patients to diminish symptoms of achy joints. In any case, long-term utilization of NSAIDs have been associated with side effects normally in the gastrointestinal tract this incorporate dyspepsia, stomach bleeding and GI perforations. It might likewise create renal impairment, for example, liver cirrhosis. All of the mentioned side effects are because of its non-selective inhibition of both constitutive (COX-1) and inducible (COX-2) isoforms of cyclooxygenase enzymes (Nigam, 1976).

The researchers, therefore, aimed to find out if the combination of Sampa-Sampalukan (*Phyllanthus niruri*) *and* Makahiya (*Mimosa pudica*) ethanolic stem extracts effectively possess anti-inflammatory activity. This study solely focused on the determination of anti-inflammatory activity of ethanolic stem extracts of the combined plants.

Research Questions

This study aimed to determine the anti-inflammatory activity of the ethanolic stem extracts of the combined Sampa-Sampalukan (*P. niruri*) *and* Makahiya (*M. pudica*) plants. Specifically, it aimed to answer the following questions:

1. What are the phytochemical constituents present in the crude Sampa-Sampalukan (*Phyllanthus niruri*) *and* Makahiya (*M. pudica*) ethanolic stem extract?
2. What is the degree of paw edema/ inflammation as evidenced by the volume of water displacement of the different treatment groups?
    a. Post-induction of Carrageenan
    b. 1 hour post-treatment
    c. 2 hours post treatment

d. 3 hours post treatment
e. 4 hours post-tretament

2. Is there a significant difference in degree of inflammation of subjects after induction of inflammation and after 1, 2, 3 and 4 hours post-treatment?
3. Is there a significant difference in the degree of inflammation of the different treatment groups 1, 2, 3 and 4 hours post-treatment?
    a) Negative Control (Distilled Water)
    b) Positive Control (Mefenamic Acid)
    c) Sampa-Sampalukan (*P.niruri*)
    d) Makahiya (*M. pudica*)
    e) Combined Sampa-Sampalukan (*P. niruri*) and Makahiya (*M. pudica*)

Hypotheses

1. There is no significant difference in degree of inflammation of subjects after induction of inflammation and after 1, 2, 3 and 4 hours post-treatment
2. There is no significant difference in the degree of inflammation of the different treatment groups 1, 2, 3 and 4 hours post-treatment.

Significance of the Study

This study is beneficial to the community through increasing awareness with the importance of herbal plants as an alternative management for inflammation exhibited by the combination of Sampa-Sampalukan *(P. niruri*) and Makahiya (*M. pudica*) stem extracts. It is beneficial to the future researchers who wish to embark on the same line of study.

Literature Review

Ethnobotany of Sampa-Sampalukan (*Phyllanthus niruri*)

Phyllanthus niruri L. (P. niruri) is called Sampa-Sampalukan. It is typically seen at roadside and garden weed (Paithankur, Raut, Charde & Vyas, 2011). It is a branching herb with little elongated stems and fruits in its branches. It is overlooked in light of the fact that it is simply viewed as a weed in parts and roadsides. It has pharmacological exercises like antimicrobial, cancer prevention agent, anticancer, mitigating, against plasmodial, antiviral, diuretics and hepatoprotective (Navneet, Balijinder, & Geetika, 2017). In the Philippines Sampa-Sampalukan is utilized as folkloric drug for kidney stones and gallstones. Decoction of whole plant fills in as tonic for the stomach, intense bitter fruit used for tubercular ulcers, wounds, injuries, scabies, and ringworm, new root utilized as solution for jaundice, additionally utilized for genitourinary issues: renal colic, cystitis, prostate issues, clogging, dyspepsia, gonorrhea, youthful stems utilized for fevers, biting of new stems utilized for hiccups, utilized for showers in babies, as emenagogue, decoction utilized for coughs in infant and infusion of root and stems utilized as tonic and taken cold in

repeated doses.

Makahiya (*Mimosa pudica*)

Mimosa pudica Linn. is common and abundant, furthermore called as Makahiya. This plant is simply viewed as a hazard in gardens, henceforth being evacuated and disposed of. Makahiya flourishes in any sort of soil even without getting uncommon care. Alkaloids, renins, saponins and tannins are available in stem. Likewise, inactive or active like starches, basic oil, flavonoids and other similar secondary metabolites are available in the entire plant. Roots are utilized as diuretics, dysmenorrhea, hostile to asthmatic, sexual enhancer, likewise for urinary complaints and powerful against looseness of the bowels (Hafsa, Saksh, Anurag, & Raji, 2012). At the point when the plant is as yet youthful, its stem is erect however as the plant develops with age, the stem moves toward becoming creeping or trailing. The stem is slim, branching and inadequately to thickly thorny, developing to a length of 1.5 meter. The stems are bipinnately compound, with maybe a couple pinnae sets having 10-26 stemlets per pinna. The petioles are additionally thorny. Pediculate (stalked) pale pink or purple blossom heads emerge from the stem axils. The globes are 8-10 mm in measurement (barring the stamens. On close examination, it is seen that the floret petals are red in their upper part and the fibers are pink to lavender. The fruits comprise of 2-8 units from 1-2 cm long each. The flowers are pollinated by the wind and insects (Racadio, 2016).

Inflammation

Inflammation is an ordinary defensive reaction to tissue damage and it includes a complex array of enzyme activation, mediator release discharge, liquid extravasations, cell relocation, tissue breakdown and repair (Vane et al., 1995). It is a complex procedure, which is much of the time related with pain and includes events, for example, the expansion in vascular penetrability, increase of protein denaturation and membrane changes (Umapathy et al., 2010). Unsafe stimuli including pathogens,

Aggravations or harmed cells start reaction of vascular tissue as inflammation. Inflammation is a defensive endeavor by the creature to expel damaging stimuli and start of healing process for the tissue (Denko.1992). However, if inflammation is not treated it leads to onset of diseases like vasomotor rhinorrhoea, rheumatoid arthritis and atherosclerosis (Henson et al., 1989). In valuing the inflammatory process, it is essential to comprehend the role of chemical mediators. These are substances that have a tendency to coordinate the inflammatory response. These inflammatory mediators go between from plasma proteins or cells including mast cells, platelets, neutrophils and monocytes/macrophages. They are activated by bacterial items or host proteins. Chemical mediators bind to particular receptors vascular permeability, neutrophil chemotaxis, stimulate smooth muscle constriction, have coordinate enzymatic action ,initiate pain or mediate oxidative harm. Most

mediators are short - lived however cause destructive impacts. Cases of chemical meditors incorporate vasoactive amines (histamine, serotonin), arachidonic acids (prostaglandins, leukotrienes) and cytokines (tumor rot factor and interleukin - 1) (Smith et al., 2004).

Synergism of Herbal Plants

Herbal medicines comprise of numerous components those varying and distinct pharmacological exercises. Particular measures of the components exhibit in the plant have an alternate pharmacological action. One component often affects other active agents pharmacologically, maybe applying what has been named a synergistic response. Synergism of herbal plants it refers to the possibility that specific parts in a herbal component can enhance the therapeutic effect of active agents, one herb can improve the effect of another given at the same time or it can likewise imply that the joined impact of various herbal components is really more greater than sum of each of the individual components. At the point when herbal plants are combined, the resulting effect is generally greater than taking alone. This is normally in view of broad experience and observation going back to numerous years. The desired result, at last, is enhanced patient advantage. Another favorable combination of herbal treatment is adjustment to healthcare escalates; bringing way of life changes is an extremely cost-effective method for expanding the likelihood of a successful remedial result. There are two principle forms by which synergy shows: Pharmacokinetic collaboration between the diverse herbs exhibit in the formulation. Pharmacokinetic interactions are those between herbal components in terms of absorption, distribution, metabolism and excretion. Pharmacodynamic interaction, this happens, for instance, when a few parts in the herbal components in the herbal medicine compete for the same physiological system or receptor. The synergistic impacts of herbal medicine likewise stretch out to their treatment of complex issue. Synergistic activity in this clinical circumstance is imperative, as the disorder is so extreme or unmanageable that no single ingredient is probably going to be adequately successful (John &Rashid, 2012).

Traditional and Alternative Medicine Act of 1997

Using herbal medicine was a practice long time ago. The community utilizes their available resources in order to prevent and manage their diseases such as infections. Herbal medications are not only considered for their effective and cost-effective way of preventing or managing infections, but also, due to their constant availability in the community. Republic Act 8423 (RA 8423) also known as the Traditional and Alternative Medicine Act of 1997, focuses on developing different traditional health-related management in the country. Drugs for prevention, cure, lessening signs and symptoms, diagnosis and maintaining a healthy lifestyle with lower price are needed to explored and developed. The alternative medications undergo methods of proper compounding (Nolledo, 2015).

This law encourages the indigenous people to share their traditional medicines and for people to study more about the safety and effectiveness of these alternative medicines. The health care professionals should become aware of these alternative medications and promote to their patients. By this, our countrymen would encounter more alternative medicines coming from that cost much lesser that existing drugs. The cheaper the medicines get, the more patients will comply with medication (Nolledo, 2015).

Research Paradigm

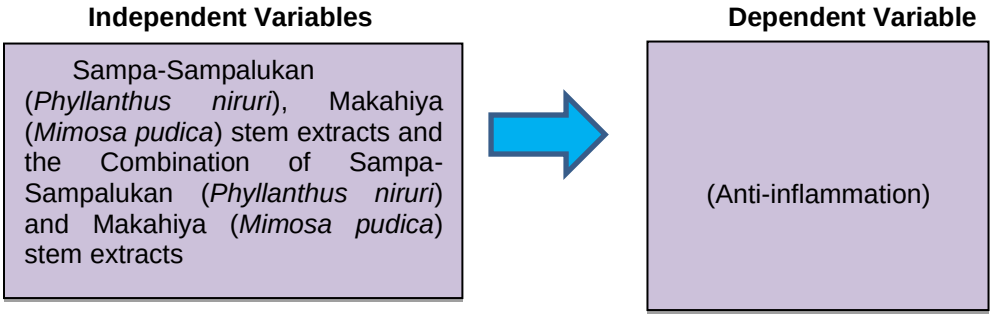

Figure 1. *Research Simulacrum*

The figure above shows the effects of Sampa-Sampalukan (P. niruri), Makahiya (*M. pudica*) stem extracts and the Combination of Sampa-Sampalukan (P. niruri) and Makahiya (*M. pudica*) stem extracts and Mefenamic Acid Suspension on the degree of inflammation in male wistar rats and how the degree of inflammation responded to the independent variables.

METHODS

Research Design

This study made use of the experimental method which included the administration of different controls in male Wistar rats and degree of inflammation in each rat has likewise been observed. The tests were directed at Philippine Institute of Traditional and Alternative Health Care.

Samples of the Study

The Sampa-Sampalukan *(P. niruri)* and Makahiya *(M. pudica*) plants were both gathered in Tuguegarao City, Cagayan.

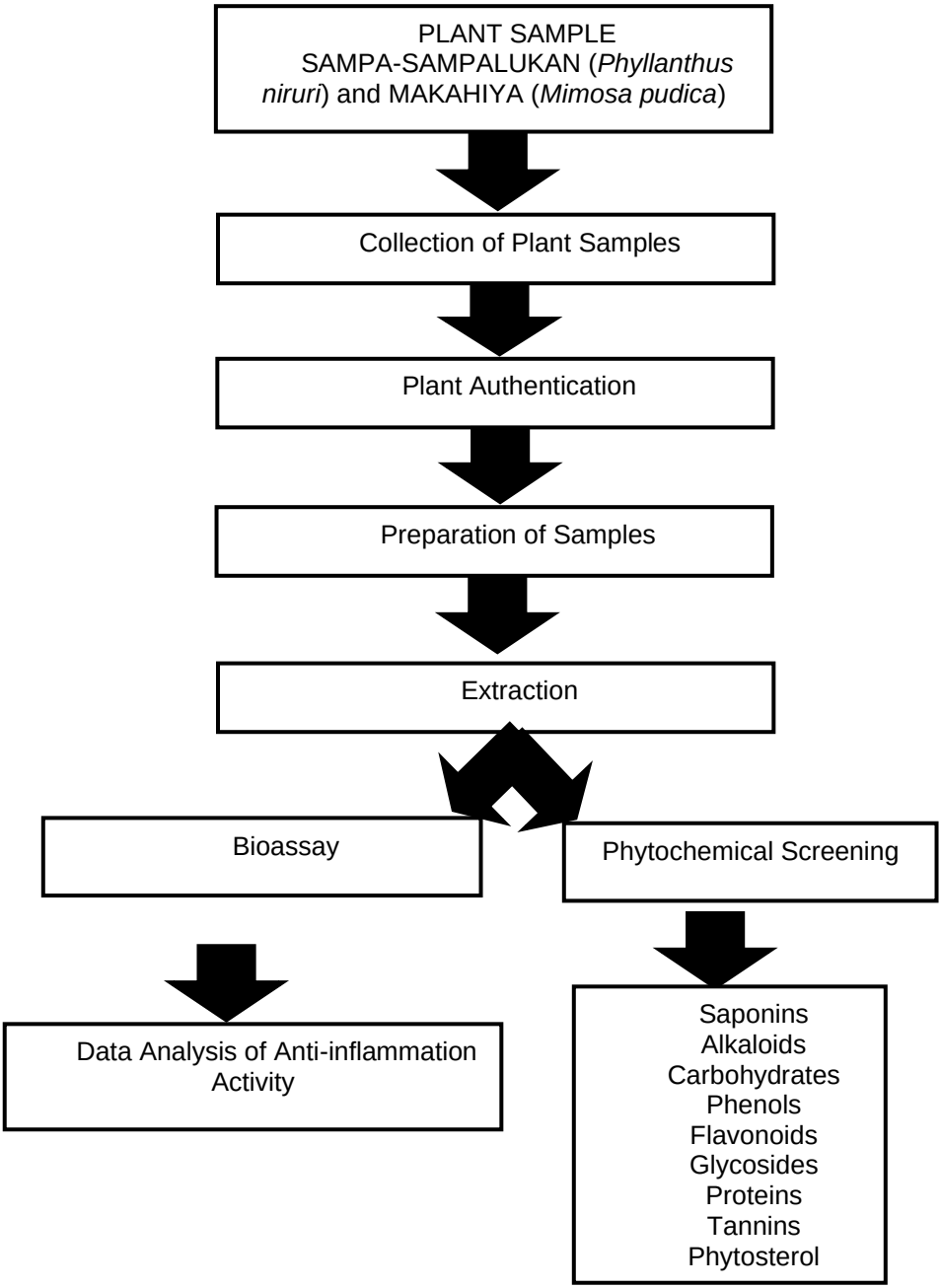

Figure 2. *Methodological Framework*

Procedures of Data Gathering

1. Collection of plant sample

1.1. The researchers collected the *Sampa-Sampalukan (P. niruri) and Makahiya (M. pudica*) plants in Tuguegarao City, Cagayan.

2. Plant authentication

2.1. The botanical verification and the authentication of the plant materials were conducted at the Department of Agriculture Bureau of Plant Industry, Carig Sur, Tuguegarao City, Cagayan.

3. Preparation of samples

3.1 The researchers removed the leaves and roots of the two plants and collected the stems. The obtained stems were then washed using tap water.
3.2 The stems of the two plants were shade dried for about 20 days in a room temperature.
3.3 The dried stems were cut into small pieces to reduce its size.
3.4 100g of each dried stems was macerated using 95% ethanol in 10:1 (v/w) for 72 hours in a 500mL Erlenmeyer flask and stored in room temperature.

4. Extraction Method

4.1 Filtration

4.1.1 After three days, the two macerated stems were filtered using filter paper and funnel. The filtrates were kept in a tight stoppered container to avoid evaporation.

5. Phytochemical Screening

5.1.1 The stems of the two plant sample were submitted to NSRU (Natural Sciences Research Unit), Saint Louis Baguio for the determination of the secondary metabolites present.
5.1.2 **Detection of saponins**. Foam Test: 1ml extract was treated with 1% lead acetate solution. Formation of white precipitates indicates the presence of saponins.
5.1.3 **Detection of flavonoids**. Lead Acetate Test: The extract was treated with a few drops of lead acetate solution. Formation of yellow precipitate indicates the presence of flavonoids.
5.1.4 **Detection of alkaloids**. Mayer's test: Few drops of Mayer's reagent (Potassium mercuric iodide solution) was added.

Formation of white or cream color precipitate indicates the presence of alkaloids.

5.1.5 **Detection of carbohydrates**. Molish's test: 2 drops of alcoholic α-naphthol solution in a test tube and 2 ml of concentrated sulfuric acid was added carefully along the sides of the test tubes. Formation of violet ring at the junction indicates the presence of carbohydrates.

5.1.6 **Detection of glycosides**. Kedde Test: Extract + 1 drop of alcohol + 2 drops of 3,5 dinitrobenzenzoic acid (Kedde reagent A) + NaOH (Kedde reagent B). Violet color indicates a positive result.

5.1.7 **Detection of Proteins.** Xanthroproteic test: To 2 ml of protein solution in a test tube add 10% of alkaline (NaOH) solution. Mixed and added 4-5 drops of 0.5% w/v copper sulphite (CuSO4) solution.Purplish violet colour was formed which indicated the presence of proteins.

5.1.8 **Detection of Phenolic Compound**. Ferric Chloride Test: Extract was treated with 3-4 drops of ferric chloride solution. Formation of black color indicates the presence of phenols.

5.1.9 **Detection of phytosterol.** Libermann Burchad's Test: The extract was dissolved in 2ml of acetic anhydride, heated to boiling. Cooled and then 1 ml of concentrated sulfuric acid were added. A brown ring formation at the junction and the turning of the upper layer to dark green confirmed test for the presence of phystosterol.

6. Experimental Animals and Diets

Male wistar rats weighing 125-165g were ordered from a trusted source in Santiago City, Isabela. The rats were acclimatized to laboratory conditions for one week before the start of the experiments. The housing condition of animals was under room temperature 22ºC (±3°C) with relative humidity of 30-70%. Lighting was artificial, the sequence being 12 hours of light, 12 hours of dark. The animals were supplied with standard pellet and unlimited water.

7. Anti-inflammatory Activity

7.1. Induction of Paw Edema

Healthy male wistar rats were used in the experiment. Determination of anti-inflammatory effect followed the experimental method of Winter *et al.* There were five

Groups and each subject contained six experimental rats. All groups were injected with 0.1mL of 1% Carrageenan suspension in the sub-plantar region, right hind

paw. First group as negative control received distilled water, Second group as positive control received Mefenamic acid (Ponstan) suspension, Third group received ethanolic stem extract of Sampa-Sampalukan (*P. niruri*), Fourth group received ethanolic stem extract of Makahiya (*M. pudica*), Fifth group received combined ethanolic stem extract of Sampa-Sampalukan (*P. niruri*) and Makahiya (*M. pudica*). The experimental rats were starved overnight with adequate supply of water prior to experimentation. Before one hour of 0.1mL of 1% Carrageenan injection, the rats were pre-treated with the negative control, positive control and experimental controls.

7.2. Measurement of Paw Edema

The measurement of paw edema was measured by volume displacement method immediately after 1% Carrageenan injection at 1,2,3,4, hours.

Ethical Consideration

For proper disposal of experimental rats that were used in the study, the researchers surrendered the experimental rats in the Philippine Institute of Traditional and Alternative Health Care (PITAHC), Carig Sur, Tuguegarao City, Cagayan.

Also, the study underwent for approval of research procedures of the University Research Ethics Board.

RESULTS

Table 1.1. *Phytochemical screening of Sampa-Sampalukan (P. niruri)*

Constituents	Description	Results
Saponins	Formation of white precipitates	Negative
Alkaloids	Formation of white or cream color precipitate	Positive
Carbohydrate	Formation of violet ring at the junction	Positive
Flavonoids	Formation of yellow precipitate	Positive
Phenols	Formation of black color	Negative
Glycosides	Violet color	Negative
Proteins	purplish violet colour formation	Positive
Tannins	Formation of white precipitate	Positive
Phytosterol	A brown ring formation at the junction and the turning of the upper layer to dark green	Positive

The table shows the presence of Alkaloids, Carbohydrates, Flavonoids, Proteins, Tannins and Phytosterol.

Table 1.2. *Phytochemical screening of Makahiya (M. pudica)*

Constituents	Description	Results
Saponins	Formation of white precipitates	Negative
Alkaloids	Formation of white or cream color precipitate	Positive
Carbohydrate	Formation of violet ring at the junction	Positive
Flavonoids	Formation of yellow precipitate	Positive
Phenols	Formation of black color	Negative
Glycosides	Violet color	Negative
Proteins	purplish violet colour formation	Positive
Tannins	Formation of white precipitate	Negative
Phytosterol	A brown ring formation at the junction and the turning of the upper layer to dark green	Positive

The table shows the presence of Alkaloids, Carbohydrates, Flavonoids, Proteins, Tannins and Phytosterol

Table 2. *Degree of Paw Edema/ Inflammation (Volume of Water Displacement) of the Different Treatment Groups Pre and Post-Treatment*

Treatment Groups	Before Induction of Edema (Mean)	After Induction of Edema (Mean)	1 Hour (Mean)	2 Hours (Mean)	3 Hours (Mean)	4 Hours (Mean)
Negative control (plain water)	0.4983	1.0883	1.0650	1.1117	1.1433	1.1800
Positive (Mefenamic Acid)	0.5117	1.0017	.9267	.9517	.8767	.8250
Experimental group 1 (Sampa-sampalukan Extract)	0.5800	1.0433	1.0083	1.0217	.9433	.8767
Experimental group 2 (Makahiya Extract)	0.5417	1.0200	.9850	1.0033	.9367	.8717

Experimental group 3 (Combined)	0.5317	1.0117	.9700	.9883	.9117	.8533

Table 2 reveals that the subjects in the negative and positive control as well as the experimental groups showed a decreasing paw edema in relation to the time after the treatments were administered.

Table 3.1. *Test of Significant Difference of the Degree of Inflammation of Subjects under the Negative Control Treatment after Induction of Inflammation and Post-treatment*

Pairs	t-value	p-value	Decision
Post-Carrageenan Administration-1 hour Post Treatment	-5.534	.003	Reject Ho
Post-Carrageenan Administration-3 hours Post Treatment	-5.534	.003	Reject Ho
Post-Carrageenan Administration-5 hours Post-Treatment	-9.774	.000	Reject Ho
Post-Carrageenan Administration-4 hours Post Treatment	-55.000	.000	Reject Ho

The table shows that the degree of paw edema or inflammation of the test subjects changed significantly after 1, 2, 3 and 4 hours of administering sterile water. However, it can be noted that there was significant increase in inflammation post administration of the negative control.

Table 3.2. *Test of Significant Difference of the Degree of Inflammation of Subjects under the Positive Control Treatment after Induction of Inflammation and Post-treatment*

Pairs	t-value	p-value	Decision
Post-Carrageenan Administration-1 hour Post Treatment	5.616	.002	Reject Ho
Post-Carrageenan Administration-3 hours Post Treatment	4.129	.009	Reject Ho
Post-Carrageenan Administration-5 hours Post-Treatment	11.829	.000	Reject Ho
Post-Carrageenan Administration-4 hours Post Treatment	20.032	.000	Reject Ho

It can be gleaned on the table above that the degree of paw edema or inflammation of the test subjects significantly decreased after 1, 2, 3 and 4 hours of administering Mefenamic Acid suspension.

Table 3.3. *Test of Significant Difference of the Degree of Inflammation of Subjects under the Experimental Group 1 after Induction of Inflammation and Post-treatment*

Pairs	t-value	p-value	Decision
Post-Carrageenan Administration- 1 hour Post Treatment	2.976	.031	Reject Ho
Post-Carrageenan Administration- 3 hours Post Treatment	3.606	.015	Reject Ho
Post-Carrageenan Administration- 5 hours Post-Treatment	7.746	.001	Reject Ho
Post-Carrageenan Administration-4 hours Post Treatment	11.294	.000	Reject Ho

It can be gleaned on the table above that the degree of paw edema or inflammation of the test subjects significantly decreased after 1, 2, 3 and 4 hours of administering the *P. niruri* stem extract.

Table 3.4. *Test of Significant Difference of the Degree of Inflammation of Subjects under the Experimental Group 2 after Induction of Inflammation and Post-treatment*

Pairs	t-value	p-value	Decision
Post-Carrageenan Administration- 1 hour Post Treatment	15.652	.000	Reject Ho
Post-Carrageenan Administration- 3 hours Post Treatment	5.000	.004	Reject Ho
Post-Carrageenan Administration- 5 hours Post-Treatment	9.882	.000	Reject Ho
Post-Carrageenan Administration-4 hours Post Treatment	13.766	.000	Reject Ho

It can be gleaned on the table above that the degree of paw edema or inflammation of the test subjects significantly decreased after 1, 2, 3 and 4 hours of administering the *M. pudica* stem extract.

Table 3.5. *Test of Significant Difference of the Degree of Inflammation of Subjects under the Experimental Group 3 after Induction of Inflammation and Post-treatment*

Pairs	t-value	p-value	Decision
Post-Carrageenan Administration- 1 hour Post Treatment	13.558	.000	Reject Ho
Post-Carrageenan Administration- 3 hours Post Treatment	11.068	.000	Reject Ho
Post-Carrageenan Administration- 5 hours Post-Treatment	12.910	.000	Reject Ho
Post-Carrageenan Administration-4 hours Post Treatment	10.304	.000	Reject Ho

It can be gleaned on the table above that the degree of paw edema or inflammation of the test subjects significantly decreased after 1, 2, 3 and 4 hours of administering the combined *P. niruri* and *M. pudica* stem extract.

Table 4. *Test of Significant Difference in the Degree of Inflammation of the Different Treatment Groups 1, 2, 3, and 4 hours Post-treatment*

Pairs	F-value	p-value	Decision
1 hour Post-treatment	4.029	.012	Reject Ho
2 hours Post-treatment	6.280	.001	Reject Ho
3 hours Post-treatment	18.870	.000	Reject Ho
4 hours Post-treatment	54.292	.000	Reject Ho

The table above shows that there is a significant difference in the degree of paw edema/ inflammation among the different treatment groups (negative control, positive control, experimental groups 1, 2 and 3) after administration of the respective treatments for 1, 2, 3 and 4 hours.

Table 5.1. *Post-Hoc Analysis of the Test of Significant Difference in the Degree of Inflammation of the Different Treatment Groups 1 hour Post-treatment*

	Mean	Negative Control	Positive control	Exp. Group 1	Exp. Group 2	Exp. Group 3
Negative control	1.0650					
Positive Control	.9267	.001 *				
Exp. Group 1	1.0083	.127	.032 *			
Exp. Group 2	.9850	.035 *	.117	.522		
Exp. Group 3	.9700	.014 *	.239	.296	.680	

**The mean difference is significant at the 0.05 level*

The table above shows that after 1 hour of treatment, the *M. pudica* extract and the combined stem extract manifested significantly the same anti-inflammatory effect as the positive control (Mefenamic Acid). Moreover, there is no significant difference in the anti-inflammatory effect of the combined stem extract and the *M. pudica* stem extract.

Table 5.2. *Post-Hoc Analysis of the Test of Significant Difference in the Degree of Inflammation of the Different Treatment Groups 2 hours Post-treatment*

	Mean	Negative Control	Positive control	Exp. Group 1	Exp. Group 2	Exp. Group 3
Negative control	1.1117					
Positive Control	.9517	.000*				
Exp. Group 1	1.0217	.013*	.048*			
Exp. Group 2	1.0033	.004*	.138	.591		
Exp. Group 3	.9883	.001*	.287	.332	.660	

**The mean difference is significant at the 0.05 level*

The table above shows that after 2 hours of treatment, the *M. pudica* extract and the combined stem extract manifested significantly the same anti-inflammatory effect as the positive control (Mefenamic Acid). Moreover, there is no significant difference in the anti-inflammatory effect of the combined stem extract and the *M. pudica* stem extract.

Table 5.3. *Post-Hoc Analysis of the Test of Significant Difference in the Degree of Inflammation of the Different Treatment Groups 3 hours Post-treatment*

	Mean	Negative Control	Positive control	Exp. Group 1	Exp. Group 2	Exp. Group 3
Negative control	1.1433					
Positive Control	.8767	.000*				
Exp. Group 1	.9433	.000*	.061			
Exp. Group 2	.9367	.000*	.090	.846		
Exp. Group 3	.9117	.000*	.313	.361	.469	

**The mean difference is significant at the 0.05 level*

The table above shows that after 3 hours of treatment, the *M. pudica* extract, *P. niruri* extract and the combined stem extract manifested significantly the same anti-inflammatory effect as the positive control (Mefenamic Acid). Moreover, there is no significant difference in the anti-inflammatory effect of the combined stem extract from the *M. pudica* stem extract and *P. niruri* extract.

Table 5.4. *Post-Hoc Analysis of the Test of Significant Difference in the Degree of Inflammation of the Different Treatment Groups 4 hours Post-treatment*

	Mean	Negative Control	Positive control	Exp. Group 1	Exp. Group 2	Exp. Group 3
Negative control	1.1800					
Positive Control	.8250	.000*				
Exp. Group 1	.8767	.000*	.077			
Exp. Group 2	.8717	.000*	.108	.860		
Exp. Group 3	.8533	.000*	.322	.413	.519	

**The mean difference is significant at the 0.05 level*

The table above shows that after 3 hours of treatment, the *M. pudica* extract, *P. niruri* extract and the combined stem extract manifested significantly the same anti-inflammatory effect as the positive control (Mefenamic Acid). Moreover, there is no significant difference in the anti-inflammatory effect of the combined stem extract from the *M. pudica* stem extract and *P. niruri* extract.

DISCUSSION

The research study aimed to determine the anti-inflammatory activity of the combined Sampa-Sampalukan (*P. niruri*) *and* Makahiya (*M. pudica*) ethanolic stem extracts. To achieve the objective of the study, phytochemical screening and biological assay (Carrageenan-induced paw edema) was conducted. The qualitative phytochemical screening of the stem extracts of both plants revealed the presence of flavonoids.

All groups showed an increase of edema at the baseline and a decrease on the 1st hour and an increased at the 2nd hour of observation due to biphasic event of Carrageenan. The first phase (0-1 hour) began after immediate injection of 0.1mL of 1% Carrageenan because of the release of histamine, serotonin and bradykinin diminished after an hour. (Crunkhorn & Meacock, 1971; Vinegar et. al, 1969) The second phase began after the first phase which increased at the 2nd hour and remained up to 4th hour because of the release of prostaglandins, protease and lysosomes (Di et. al, 191; Halici et. al., 2007; Virheva et. al, 2011).

Mefenamic Acid Suspension (Ponstan) was used as a reference drug to compare the anti-inflammatory effect of Sampa-Sampalukan (*P. niruri*), Makahiya (*M. pudica*) and Combination of Sampa-Sampalukan (*P. niruri*), Makahiya (*M. pudica*). The result showed that the three treatments had no significant difference

with the Mefenamic acid Suspension which means that they are as effective as the Mefenamic Acid Suspension. Also, statistical analysis showed that there is a significant difference before and after administration of the treatments and in the size of the paw after administration of 1% Carrageenan in pre-treated rats.The decrease of paw volume on every hour of observation is shown in the Positive Control, Sampa-Sampalukan (*P. niruri*), Makahiya (*M. pudica*) and the Combination of Sampa-Sampalukan (*P. niruri*) and Makahiya (*M. pudica*) thus, it justifies that the combination of Sampa-Sampalukan (*P. niruri*) and Makahiya (*M. pudica*) stem extracts can be used as an alternative for the treatment of inflammation.

CONCLUSION

Based on the findings of the researchers, the following conclusions were made for the determination of the anti-inflammatory of combination of Sampa-Sampalukan (*P. niruri*) and Makahiya (*M. pudica*) ethanolic stem extracts exerts an anti-inflammatory activity and it is as effective as Positive control Mefenamic Acid Suspension. This was proven by the data which have been collected and justified by the statistical analysis of this research. However, the combination of the two plant extracts proved to have the same anti-inflammatory effect as when these extracts are used individually.

RECOMMENDATION

The researchers would highly recommend the following:

1. Do perform histopathology to determine if the Combination of Sampa-sampalukan (*P. niruri*) and Makahiya (*M. pudica*) stem ethanolic extracts causes' damage renal function and gastro-ulcerations.
2. Do use the standard equipment such as Plethysmometer in anti-inflammatory study to lessen the time and burden in measuring as compared to volume displacement method.
3. Do have further researches about the different concentrations to determine the most effective dose that exerts anti-inflammatory activity.
4. Do include quantitative phytochemical analysis.

REFERENCES

Aldini, R., Micucci, M., Cevenini, M., Fato, R., Bergamini, C., Nanni, C., ... & Roda, G. (2014). Antiinflammatory effect of phytosterols in experimental murine colitis model: prevention, induction, remission study. *PloS one*, *9*(9).

Amritpal S., Samir M., Ravi S. (2008, August 12). Anti-inflammatory and Analgesic Agents. *Indian Medicinal Plants.* Retrieved from https://pdfs.semanticscholar.org/81c7/c529d32aaaa51cfd49dc04b29858cf6e9b27.pdf

Banerjee A, Nigam SS. (1976). Antifungal activity of the essential oil of Curcuma caesia Roxb. *Indian Journal of Medical Research*. Retrieved from https://europepmc.org/abstract/med/1010624

Harsha, S. N., & Latha, B. V. (2012). In vitro antioxidant and in vitro anti inflammatory activity of Ruta graveolens methanol extract. *Asian J Pharm Clin Res*, *5*(1), 32-35. Retrieved from http://www.ajpcr.com/Vol5Issue1/621.pdf

Kaur, N., Kaur, B., & Sirhindi, G. (2017). Phytochemistry and pharmacology of Phyllanthus niruri L.: a review. *Phytotherapy Research*, *31*(7), 980-1004.

Leyva-López, N., Gutierrez-Grijalva, E. P., Ambriz-Perez, D. L., & Heredia, J. B. (2016). Flavonoids as cytokine modulators: a possible therapy for inflammation-related diseases. *International journal of molecular sciences*, *17*(6), 921.

Montejo, J. F., Mondonedo, J. A. B., Lee, M. G. A., Ples, M. B., & Vitor II, R. J. S. (2015). Hematological effects of Ipomoea batatas (camote) and Phyllanthus niruri (sampa-sampalukan) from Philippines in the ICR mice (Mus musculus). *Asian Pacific Journal of Tropical Biomedicine*, *5*(1), 29-33.

Morris, C. J. (2003). Carrageenan-induced paw edema in the rat and mouse. In *Inflammation protocols* (pp. 115-121). Humana Press.

Padmanabhan, P., & Jangle, S. N. (2012). Evaluation of in-vitro anti-inflammatory activity of herbal preparation, a combination of four medicinal plants. *International journal of basic and applied medical sciences*, *2*(1), 109-116.

Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: an overview. *Journal of nutritional science*, *5*.

Queiroz, F. M. D., Matias, K. W. D. O., Cunha, M. M. F. D., & Schwarz, A. (2013). Evaluation of (anti) genotoxic activities of Phyllanthus niruri L. in rat bone marrow using the micronucleus test. *Brazilian Journal of Pharmaceutical Sciences*, *49*(1), 135-148.

Racadio, S. P. (2016). The Medicinal Prospects of Makahiya (Mimosa Pudica Linn) Plant.Retrieved from http://article.sapub.org/10.5923.j.als.20160601.02.html

Sadeghi, H., Mostafazadeh, M., Sadeghi, H., Naderian, M., Barmak, M. J., Talebianpoor, M. S., & Mehraban, F. (2014). In vivo anti-inflammatory properties of aerial parts of Nasturtium officinale. *Pharmaceutical biology*, *52*(2), 169-174.

Souto, A. L., Tavares, J. F., Da Silva, M. S., Diniz, M. D. F. F. M., Athayde-Filho, D., Filgueiras, P., & Barbosa Filho, J. M. (2011). Anti-inflammatory activity of alkaloids: an update from 2000 to 2010. *Molecules*, *16*(10), 8515-8534.

Tag, H., Das, A. K., & Loyi, H. (2007). Anti-inflammatory plants used by the Khamti tribe of Lohit district in eastern Arunachal Pradesh India.

Umapathy, E., Ndebia, E. J., Meeme, A., Adam, B., Menziwa, P., Nkeh-Chungag, B. N., & Iputo, J. E. (2010). An experimental evaluation of Albuca setosa aqueous extract on membrane stabilization, protein denaturation and white

blood cell migration during acute inflammation. *J Med Plants Res*, *4*(9), 789-795.

Winter, C. A., Risley, E. A., & Nuss, G. W. (1962). Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. *Proceedings of the society for experimental biology and medicine*, *111*(3), 544-547.